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Inhalation and injection studies in rats using dust samples from chrysotile asbestos prepared by a wet dispersion process

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Summary. Long term inhalation studies and intraperitoneal injection studies in rats were undertaken with a series of chrysotile asbestos dusts. Three dust samples were generated from chrysotile modified by the wet dispersion process (WDC) and one was from unmodified chrysotile. Following a 1 year inhalation period, all the chrysotile samples proved extremely fibrogenic and carcinogenic and there were no significant differences between the WDC dusts and normal chrysotile. In all experimental groups approximately 25% of animals developed pulmonary carcinomas and in the oldest rats advanced interstitial fibrosis occupied on average 10% of all lung tissue. In the injection studies all the dust samples produced mesotheliomas in over 90% of animals. Very little chrysotile remained in the lungs of the animals that survived longest following dust inhalation and what there was was present as individual chrysotile fibrils. It is suggested that chrysotile is potentially the most harmful variety of asbestos as shown in these and other animal studies but that it is removed from lung tissue quite rapidly. In the long lived human species this may mean that except where exposure levels are very high and of long duration, chrysotile should be less hazardous than other asbestos types.

Keywords: Chrysotile asbestos, wet dispersion process, carcinoma, fibrosis

The industrial use of asbestos has been shown to result in considerable health risk to asbestos workers (Selikoff & Lee 1978). Heavy exposure can result in pulmonary interstitial fibrosis (asbestosis), bronchial carcinoma and mesothelioma.

While the main commercially used asbestos varieties differ markedly in their chemical composition, all show considerable potential to produce pulmonary disease when inhaled. It is now believed that the most important dust factor in disease development may be the physical dimension of the fibres, particularly length (Stanton 1972; 1977). Numerous experimental studies have been undertaken in order to examine these effects but most have used either *in vitro* or injection techniques because these require only small amounts of dust which can be specially prepared. Long-term inhalation studies unfortunately require large amounts of asbestos dust and so far it has not been possible to obtain sufficient size-selected dusts of any single asbestos type to examine the effects of fibre length when dusts are inhaled. As an alternative approach, studies are continuing in this Institute using such asbestos materials as are available that are likely to produce dust clouds of significantly different fibre dimensions. One such type of

material is chrysotile that has been treated by the 'wet dispersion' process. This process is used commercially with minor variations throughout the world and its basic principles have been described by Heron & Huggett (1971). In the wet dispersion process bulk chrysotile is treated with a dilute solution of an anionic surface active agent so that the individual chrysotile fibrils separate to produce a liquid slurry. The fibrils coagulate into a film on the application of electrolyte and this process can be controlled to spin out a yarn in which all the fibrils are intermingled and bound to one another. The yarn is then treated to remove excess surface active agent although a small amount remains bonded to the fibril surface. This is in contrast to normal chrysotile textile yarn which consists of separate chrysotile fibres, mainly parallel to one another and held together only by the spinning process. Smither & Lewinsohn (1973) described wet dispersed chrysotile yarn as being smoother, stronger and more regular than standard asbestos yarns and suggested that its use might be without the dust-related health hazards of chrysotile produced by conventional techniques. Because the chrysotile fibril arrangement in wet dispersed chrysotile yarn is so different from materials previously used in animal experimentation, it was considered that an inhalation study using this material might produce new information on the way pulmonary tissues react with different types of fibre. Parallel injection studies were conducted for closer comparison with Stanton's work.

Materials and methods

Experimental protocol for inhalation studies. Groups of 48 white SPF rats of the AF Han strain were exposed by inhalation to one of four different chrysotile preparations. These were: (a) Yarn from wet dispersed chrysotile production process. (WDC yarn); (b) Dust collected from the factory air in a workshop processing only this type of WDC (Factory WDC); (c) A standard chrysotile textile yarn produced by traditional methods (Chrysotile yarn); (d) Yarn from an experimental WDC process which was never commercialized (Exp. WDC).

Dusting was for 7 h a day, 5 days a week for a total of 224 days during a period of 12 months. One additional group of rats was exposed to dust from (d) using a reversed daylight regimen in which the rats were exposed during their period of maximum activity. This experimental variation was included because there was some evidence (Middleton *et al.* 1979) that pulmonary deposition is increased under these conditions and this could affect the development of pulmonary pathology. This dust treatment group is subsequently referred to as 'WDC Reversed Daylight'. Groups of 39 and 25 control rats were maintained for their full life span during the period of these inhalation studies.

Dust cloud generation and monitoring. Dust clouds were generated by the methods described previously by Davis *et al.* (1985*a*) and summarized in Table 1. The dust generators were able to produce satisfactory clouds from the factory WDC as supplied but dust generation from WDC yarn and Exp. WDC proved difficult and these yarns required a vigorous preliminary milling using a Christie and Norris mill. The sample of chrysotile yarn was treated in a similar manner to ensure comparability with the dusts generated from the WDC preparations. Dust concentrations in the chamber were monitored daily throughout all exposure time for mass concentration and by approximately 100 short period (snatch) samples at intervals during the exposure period for number concentration. The fibre size distributions were assessed by phase contrast optical microscopy (PCOM) for fibres >5 μm in length and both fibre length and diameter distributions were obtained by scanning electron microscopy (SEM) for fibres >0.4 μm in length and >0.1 μm in diameter.

Histopathological studies. Four animals from each inhalation group were killed at the end of the 12 month dusting period and four

Table 1. Dust sampling techniques

Type of sample	Description of sampling equipment	Sampling period	Analysis
Total dust	Open filter, 50 mm diameter sampling flow rate 2 litres/min facing downwards	The full 7 h exposure	gravimetric
Respirable dust	Casella MRE 113A gravimetric* dust sampler	The full 7 h exposure	gravimetric
	Vertical elutriator†	At least 1 full day	Infra-red spectroscopy‡
Fibre number	Open filter holder, 25 mm filter diameter in Gelman head facing downwards	Of the order of 1 min	
	sartorius membrane filter and nuclepore polycarbonate membrane filter		counting and sizing of fibres by phase contrast optical microscope§|| × 600
			sizing of fibres by electron microscopy × 10 000

* Dunmore *et al.* (1964).
† Walton (1954).
‡ Dodgson & Whittaker (1973).
§ Asbestosis Research Council (1971).
|| Walton & Beckett (1977).

more were killed 6 months later. The remaining animals were left for their full life span except that the study was terminated when the number of survivors in some groups dropped to six. Estimations of early pathological changes were limited to the small groups of animals from the first two killing dates. However, for the more advanced lesions occurring in the oldest animals, it was decided to include all those dying within 2 months of the final killing date. In practice this produced groups of animals varying from 14 to 21 in number. All control animals were allowed to survive for their full life span. Tissue used for histological examination was fixed in Karnovsky's fixative. Lungs were fixed by inflation *in situ* at a standard pressure of 30 cm of fixative. Subsequently the trachea was ligated and the lungs excised and immersed in fixative before histological processing and embedding in paraffin wax. Sections were cut in the coronal plane at 1 mm intervals and were stained by either haemotoxilin and eosin, Van Gieson's method for collagen or Gordon and Sweet's stain for reticulin. Tissue samples from some of the oldest animals were prepared for transmission electron microscopy by postfixation in a 2% solution of osmium tetroxide before embedding in araldite. Sections were stained with both lead citrate and uranyl acetate.

Measurement of pulmonary fibrosis was undertaken by similar methods to those previously published (Davis *et al.* 1978) except that an electronic image analyser (Graphic Information Systems Limited, GDS1) was available for use in conjunction with the light microscope. Single coronal lung sections were used with the section selected from the central region but avoiding the major conducting airways and pulmonary vasculature. Interstitial fibrosis was estimated using a × 2 microscope objective lens and is expressed as a percentage of total lung tissue area (Davis *et al.* 1978). Peribronchiolar lesions are more numerous and smaller and so the lung tissue was scanned with a × 4 objective, using an eyepiece graticule divided into 100 squares which at this magnification covered a tissue area of 2.9 mm^2. Peribronchiolar lesions were recorded as a percentage of squares containing lesions of this type.

Lung dust content. Lung dust estimations were performed on animals from the first two killing dates. Only the left lung was used so that the right lung was available for histological studies. Dust retained in the lungs was recovered by a low temperature plasma ashing process using a Nanotech P100 apparatus and estimated by infrared spectrophotometry (Dodgson & Whittaker 1973). Studies in this laboratory have shown that the dust content ratio between left and right lungs following experimental inhalation of fibrous dust such as asbestos in rats is 0.6:1 and this correction factor was therefore used to estimate the pulmonary dust burden of each animal.

Animal injection studies. In addition to the inhalation studies, the ability of the four chrysotile samples to produce mesothelioma was examined using the intraperitoneal injection assay. For the WDC yarn, the factory WDC and the chrysotile yarn, groups of 24 rats received a dose of 25 mg of dust. For the exp. WDC, the group size was 32 animals. The dust samples were suspended in 2 ml of Dulbecco's phosphate buffered saline and were injected into the peritoneal cavities of the rats under ether anaesthetic. The dust was collected from the animal inhalation chambers using an elutriation process in an attempt to simulate the respirable fraction of the dust clouds used in the inhalation studies (Bolton *et al.* 1982).

Results

Dust cloud generation and measurement

During development work on dust generation procedures it was found to be extremely difficult to generate high density clouds of respirable fibres from bulk prep-

Table 2. Mass and fibre number concentrations for the dust clouds generated from wet dispersed chrysotile products and standard chrysotile textile yarn

Dust Exposure	Type of measurement: Respirable dust concentration mg/m^{-3} (Means of daily estimations—see text)			Total dust concentration mg/m^{-3}	Fibre number: 'Snatch' samples on membrane filters. Counted by phase contrast optical microscopy. Mean of 100 samples
	Casella MRE 113A (incorporating a horizontal elutriator)	Vertical elutriator			
	gravimetric gravimetric	gravimetric fibres/ml	infra-red absorption spectroscopy		
WDC yarn	3.6	3.5	3.6	4.6	679
Factory WDC	3.6	3.7	2.8	4.8	468
Chrysotile yarn	3.6	3.5	3.7	4.3	428
Exp. WDC	4.4	3.5	3.3	5.7	108
Exp. WDC (Reversed daylight)	4.7	3.8	3.4	5.6	111

arations of wet dispersed chrysotile (WDC). This applied particularly to the exp. WDC sample. Even after treatment with a Christie and Norris Mill it was found that the maximum concentration of respirable dust that could be generated was approximately 4 mg/m^3 of air. It was therefore decided to undertake the present study at this dust concentration even though the results would not be directly comparable with previous long-term inhalation studies with asbestos from this Institute which had standardized on an exposure concentration of 10 mg/m^3 (Davis *et al.* 1978, 1980, 1985*a*, *b*).

The average respirable dust mass concentrations that were attained for all five exposures were close to the planned level of 4 mg/m^3. These figures together with total dust mass concentrations and fibre number concentrations are given in Table 2. The respirable mass concentration of chrysotile estimated by infrared absorption spectroscopy falls within the range 3.3–3.7 mg/m^3 for four experiments but is 2.8 mg/m^3 for the factory WDC. This sample was, however, known to contain some non chrysotile con-

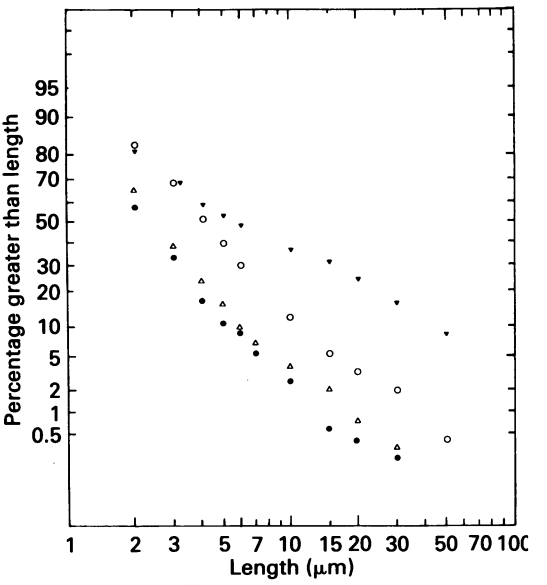

Fig 2. Length distribution of fibres longer than 0.4 μm. ○, WDC yarn; △, factory WDC; ●, chrysotile yarn; ▼, exp. WDC. Scanning electron microscope × 10000.

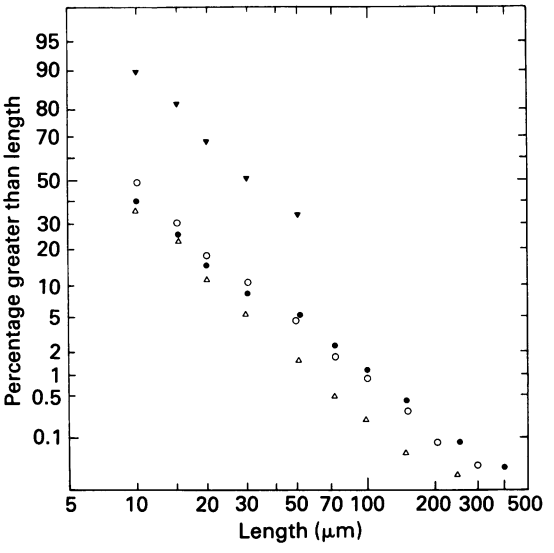

Fig 1. Length distribution of fibres longer than 5 μm ○, WDC yarn; △, factory WDC; ●, chrysotlite yarn; ▼, exp. WDC. Optical microscope × 600.

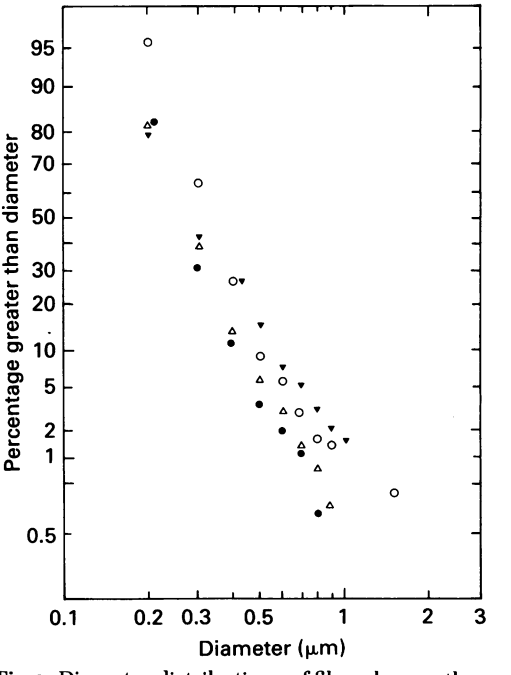

Fig 3. Diameter distributions of fibres longer than 0.4 μm ○, WDC yarn; △, factory WDC; ●, chrysotile yarn; ▼, exp. WDC. Scanning electron microscope × 10000.

taminants from the factory environment. For three of the dusts used in this study, WDC yarn, factory WDC and chrysotile yarn, the respirable mass concentrations were about 80% of the total dust concentration. For the exp. WDC, however, the equivalent value was only 60%. This indicates that the dust cloud generated from this material contained coarser fibres and this is confirmed by the number concentration and the size distribution data illustrated in Table 2 and Figs 1, 2 and 3. The number concentrations (fibres >5 μm in length) obtained by PCOM were approximately 100 fibres per ml for the exp. WDC and in the range 400–700 fibres per ml for the other clouds. Fibre length distributions from the experimental dust clouds obtained by both light microscopy (magnification $\times 600$) and SEM (magnification $\times 10\,000$) are illustrated in Figs 1 and 2 and SEM diameter distributions are given in Fig. 3. These size distributions confirm that the exp. WDC consisted of fibres that on average were much longer and slightly thicker than the other materials in the study. The fibre length distributions obtained by light microscopy suggested that the dusts from WDC yarn, factory WDC and chrysotile yarn were very similar but SEM examination showed that the WDC yarn had fibres slightly longer than the factory WDC or the chrysotile yarn.

Histopathological findings

All five groups of rats dusted with the chrysotile preparations developed the same pattern of pathological change previously reported in similar studies from this Institute. At the end of the 12 months dusting period the main lesions present were deposits of granulation tissue around the terminal and respiratory bronchioles (Figure 4). This gra-

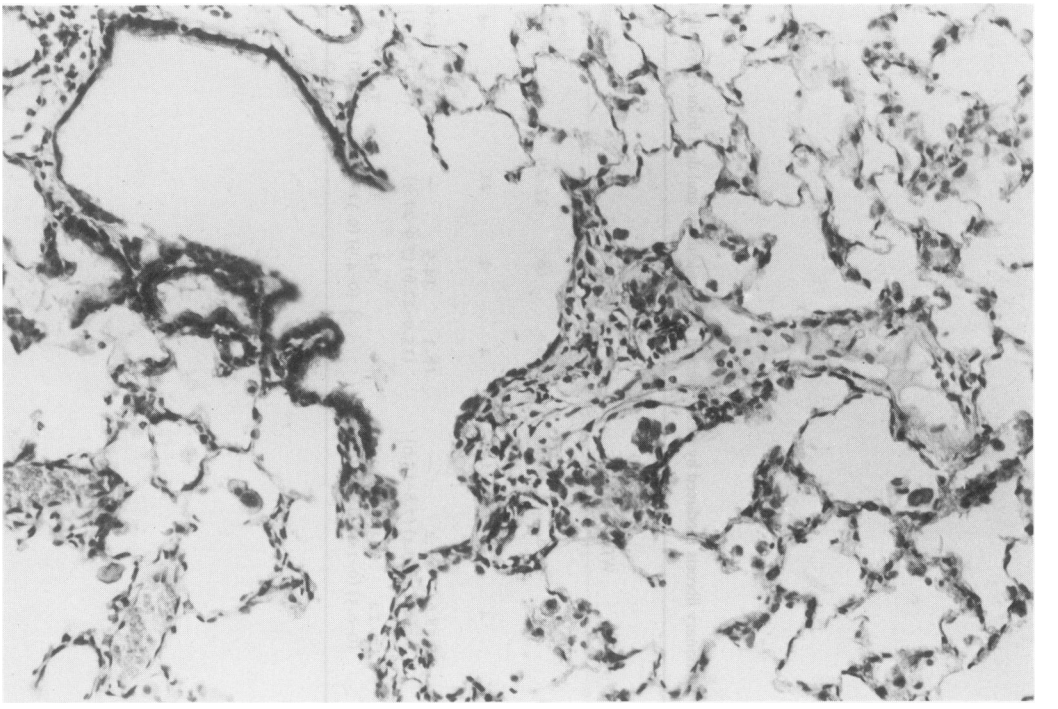

Fig 4. An area of fibrotic granulation tissue close to the terminal bronchiole of a rat treated by inhalation with dust from wet dispersed chrysotile for twelve months. $\times 320$.

Table 3. Mean levels of pulmonary fibrosis produced by wet dispersed chrysotile dusts and dust from chrysotile textile yarn (ranges in brackets)

	WDC yarn			Factory WDC			Chrysotile yarn			Exp. WDC			Exp. WDC Reversed Daylight			Controls 1	Controls 2
Time after start of exposure (months)	12	18	27–29	12	18	27–29	12	18	27–29	12	18	27–29	12	18	27–29	27–29	27–29
No. of rats examined	4	4	15	4	4	21	4	4	15	4	4	13	4	4	13	9	9
Peribronchiolar fibrosis	18.6 (17.3–20.4)	11.2 (7.8–15.0)	—	18.3 (15.9–22.6)	14.5 (7.9–21.9)	—	16.2 (11.2–21.5)	15.6 (11.6–21.5)	—	28.1 (20.8–32.3)	18.7 (11.9–28.7)	—	20.3 (13.2–26.6)	23.0 (16.0–28.8)	—	0	0
Interstitial fibrosis	0.12 (0–0.5)	0.3 (0–1.2)	12.8 (2.24–40.66)	0	1.2 (0–4.9)	12.1 (0.31–34.8)	1.2 (0–4.8)	1.1 (0–3.7)	8.8 (3.14–16.9)	0	0 (0.46–21.7)	9.6	0	0 (0.63–22.11)	10.8	0.5 (0–3.0)	0

nulation tissue consisted mainly of macrophages and fibroblasts but foreign body giant cells were also present. At 12 months after the start of dusting there was marked reticulin staining in the peribronchiolar deposits although relatively little collagen could be demonstrated by Van Gieson's stain. With increasing time after dust exposure, however, collagen staining became progressively more marked. All treatment groups showed deposits of this peribronchial fibrosis around many of the smallest airways at 12 months (Table 3) but levels in the two groups of animals treated with exp. WDC were significantly higher than for the groups treated with WDC yarn, factory WDC or chrysotile yarn. ($P = < 0.05$). By 18 months, four out of the five treatment groups showed a reduction in peribronchiolar fibrosis and taken overall, this reduction was significant ($P = < 0.05$). After 18 months from the start of dusting, widespread alveolar interstitial fibrosis developed and this tended to obscure the earlier fibrotic deposits. For this reason, estimations of peribronchiolar fibrosis were limited to the first two killing dates.

From about 18 months onwards, areas of lung tissue in some animals showed a progressive thickening of alveola septa. (Fig. 5). In its earliest form this thickening was caused almost entirely by hyperplasia of alveolar lining cells, but later there was considerable deposition of reticulin and eventually collagen in the septal walls. As shown in Table 3, areas of alveolar interstitial fibrosis became more widespread in all treatment groups with increasing time after the end of the dusting period. While the areas measured varied from a mean of 8.8% of lung tissue in animals treated with chrysotile yarn to 12.8% in animals treated with WDC yarn, these differences were not statistically

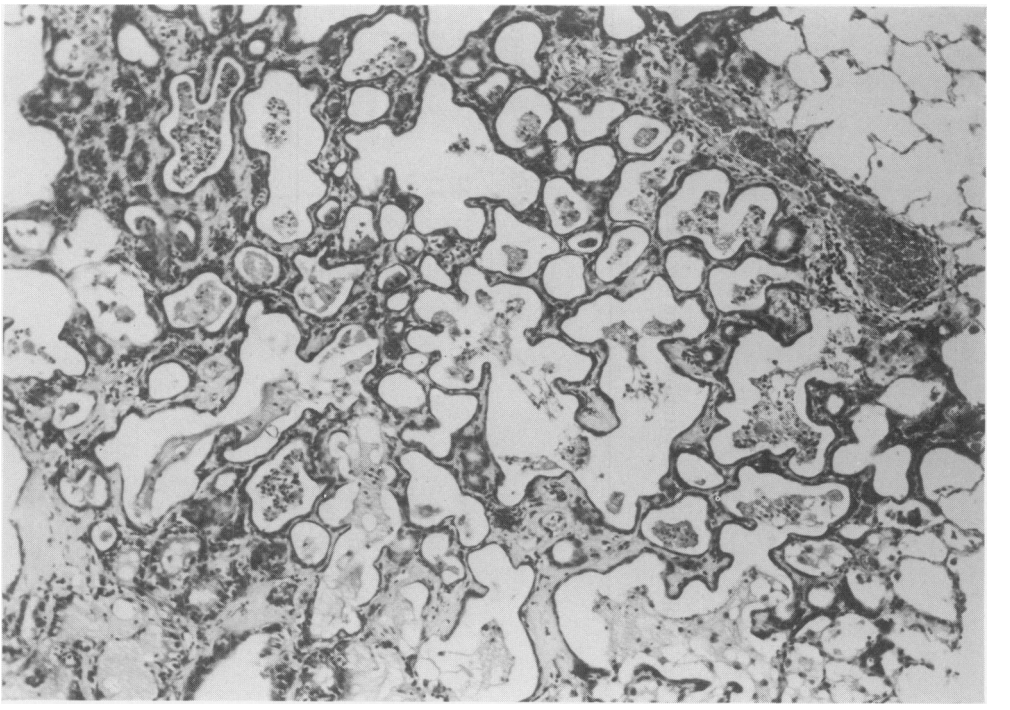

Fig 5. Alveolar interstitial fibrosis in a rat treated by inhalation with dust from wet dispersed chrysotile for 24 months. × 188.

Table 4. Pulmonary tumours and mesotheliomas produced by wet dispersed chrysotile dusts and dust from standard chrysotile textile yarn

	WDC yarn	Factory WDC	Chrysotile yarn	Exp. WDC	Exp. WDC (Reversed Daylight)	Controls 1	Controls 2
No. of rats examined	41	44	42	43	37	39	25
Adenomas	5	11	2	7	5	1	0
Total carcinomas	13	10	13	10	12	1	0
Adeno-carcinomas	7	7	9	5	5	1	0
Squamous carcinomas	6	3	4	5	7	0	0
Mesotheliomas	0	0	1	4	1	0	0

significant. In many areas of lung tissue the interstitial fibrotic element of these lesions remained predominant throughout the study, but in others the hyperplasia of alveolar epithelial cells became progressively more marked to produce a pattern of adenomatosis. Some definite adenomas could be seen to have developed from the central regions of these areas and it is likely that this was also the site of origin of some carcinomas although by the time most of these were discovered they were too widespread to be certain.

All five groups of dusted rats in this study developed primary pulmonary tumours in over 35% of animals with no significant differences between treatment groups. (Table 4). While carcinomas predominated in four of the groups, the group treated with factory WDC, which had the highest overall figures did have 11 adenomas to 10 carcinomas. One adenoma and one carcinoma were found in control animals. The carcinoma was a small lesion (2 mm diameter) which showed only early signs of invasion and was not responsible for the death of the animal.

As with other animal studies in which asbestos has been administered by inhalation, relatively few mesotheliomas developed (Table 4) but the groups of rats treated with the various chrysotile preparations all showed areas of vesicular pleural metaplasia. Histological examination showed these areas only occurred where patches of alveolar interstitial fibrosis had reached the lung surface although this pleural involvement did not always result in metaplasia. Areas of metaplasia consisted of loose fibrous tissue containing large vesicular spaces lined with flattened cells. (Fig. 6). Electron microscope studies showed that these cells were of mesothelial type and that occasionally the walls between vesicular spaces were so thin that they consisted of two closely apposed

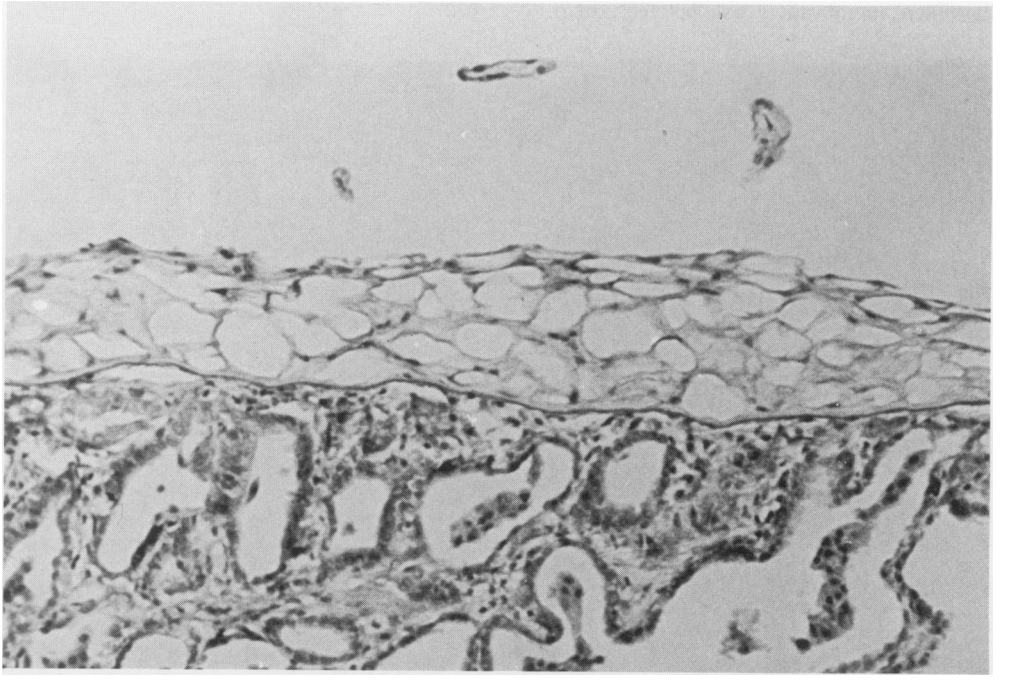

Fig. 6. Vesicular metaplasia on the pleural surface of a rat treated by inhalation with dust from wet dispersed chrysotile. The pulmonary parenchymal tissue close to the pleura shows alveolar interstitial fibrosis with the alveolar spaces lined with prominent rounded epithelial cells. × 320.

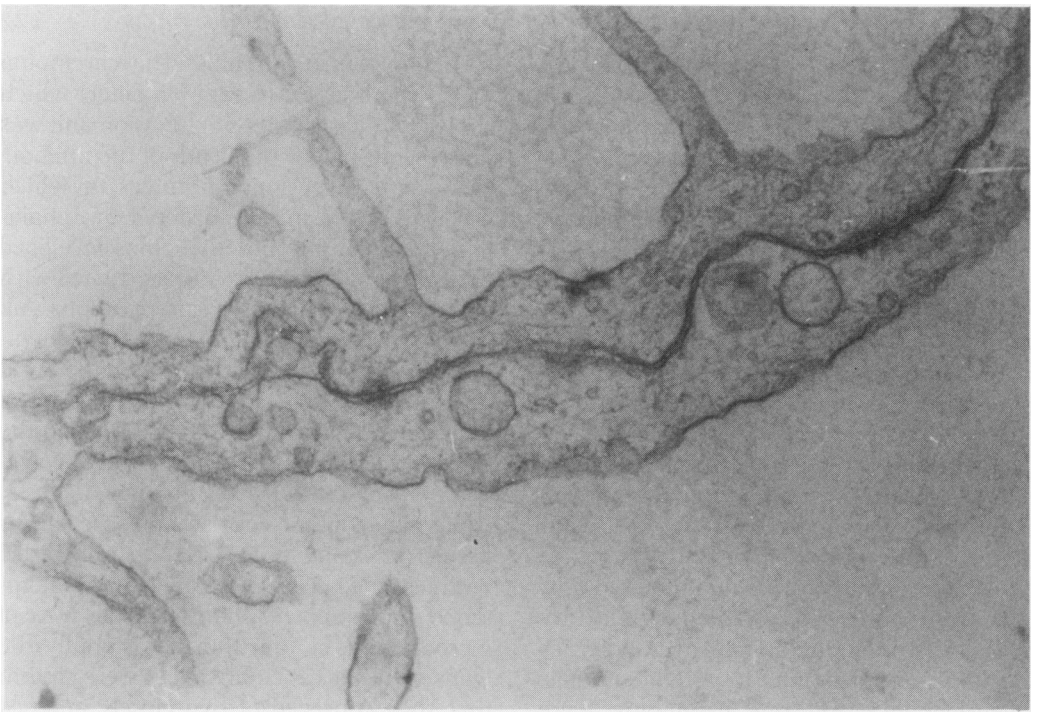

Fig 7. Transmission electron microscope photograph of the wall of one vesicle in an area of pleural metaplasia. In this case the extremely thin wall consists only of two flattened cells of mesothelial type with no basement membrane visible between them. × 32 300.

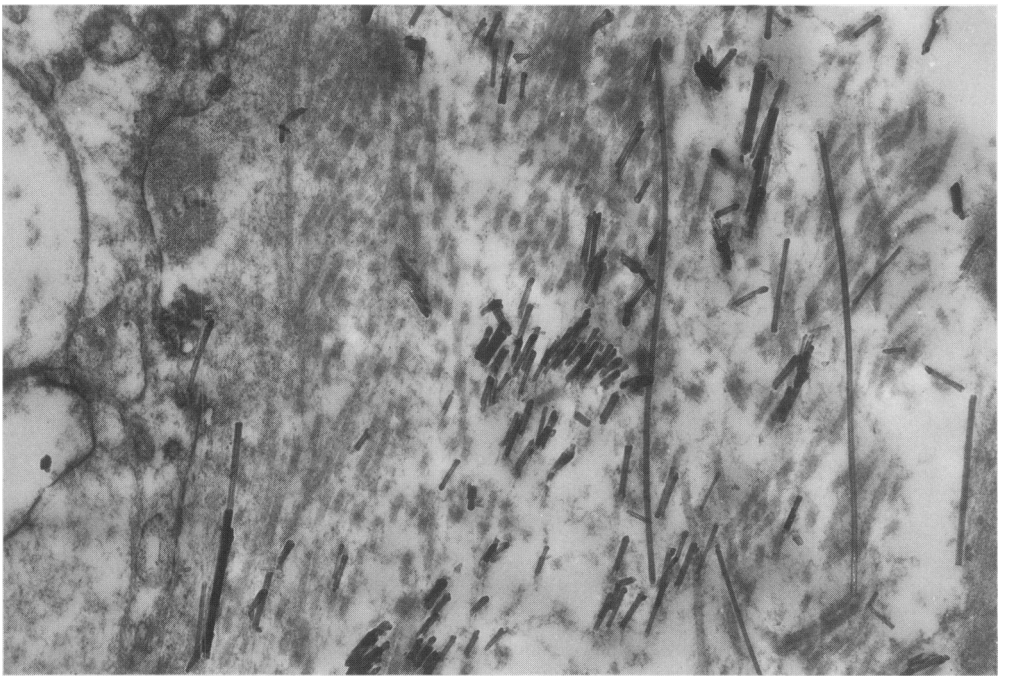

Fig 8. Transmission electron microscope photograph of an area of pulmonary fibrosis from the lung of a rat twenty seven months after the start of treatment with dust from wet dispersed chrysotile. Numerous individual chrysotile fibrils are visible among the collagen fibres, the longest chrysotile fibril in this photograph being over 5 μm in length. × 36 000.

Table 5. Numbers of tumours occurring at sites other than lung

	WDC yarn		Factory WDC		Chrysotile yarn		Exp. WDC		Exp. WDC (Reversed Daylight)		Controls 1		Controls 2	
Number of rats examined	41		44		42		43		37		39		25	
Organ System	B	M	B	M	B	M	B	M	B	M	B	M	B	M
Digestive/peritoneal	1	1		2		2	1	3	1		1	3	1	
Urinogenital		1	1	1	1		3			1		2	2	
Endocrine	4	1	1	1	3	1	3		1	1	6	2	3	
Musculo, skeletal and integumentary	1	2	1	2	1	4	5	1	4	4		5	2	3
Reticuloendothelial/ vascular		1		3		3	0	1		2		3		1
Totals	6	6	3	9	5	10	12	5	6	8	7	15	8	4

B, benign; M, malignant.

layers of extremely extended and flattened cells with no basement membrane. (Fig. 7). Where cells were supported by areas of fibrosis a basement membrane was present.

Electron microscope examination of specimens of lung parenchyma from some of the animals in the present study, several months after the end of dusting showed that most of the original chrysotile fibre bundles had separated into individual fibrils. This was particularly marked with the preparations of WDC (Fig. 8) but was also apparent in tissues from animals treated with dust from the standard chrysotile yarn. In the animals that survived longest after the end of dust exposure, only small amounts of chrysotile remained in the lung tissue and this was almost entirely confined to two tissue sites. These were firstly, areas of fibrosis, where chrysotile fibrils could be found amongst the collagen fibres and secondly, in the alveolar basement membranes, which were often greatly thickened. Almost no chrysotile was found in alveolar macrophages in the oldest rats from the present study.

Apart from the pulmonary tissue both the five treatment groups of rats and the two control groups progressively showed the normal types of pathological change associated with advanced age. The most common lesion in this category was chronic progressive nephrosis which was found in over 50% of animals. The numbers of non-pulmonary tumours found in the present study are listed in Table 5. There were no significant differences between the five dust exposure groups. One batch of controls did have noticeably more tumours than chrysotile treated animals but since this was not apparent in the other control group it is likely to have been due to chance.

Lung dust burden

The retained lung burden of chrysotile dust at the end of a dusting period and six months later is illustrated in Table 6 for all treatment groups. At the end of dusting the mean figures ranged between 250 μg and 500 μg for the five groups while 6 months later between 15% and 60% of this dust had been cleared. The lung dust samples from one group of animals killed at 18 months after treatment with the exp. WDC were accidentally destroyed during analysis.

Injection studies

All the four samples of chrysotile under consideration produced mesotheliomas in over 90% of rats following the intra-peritoneal injection of 25 mg of dust. A difference was apparent, however, in the mean tumour induction period. While the WDC yarn, factory WDC and exp. WDC all produced mesotheliomas in an average of 310–340 days, the standard chrysotile yarn required an average of 400.

Table 6. Mean masses of chrysotile dust receovered from rat lung tissue

Dust type	Time after the end of dusting	
	3 days	6 months
WDC yarn	420 μg (120)	150 μg (55)
Factory WDC	240 μg (8)	120 μg (33)
Chrysotile yarn	400 μg (94)	230 μg (49)
Exp. WDC	490 μg (78)	—
Exp. WDC (Reversed daylight)	280 μg (25)	240 μg (68)

Figures in brackets are standard deviations.

Discussion

The three samples of wet dispersed chrysotile and one sample of dust from normal chrysotile textile yarn examined in the present study all proved to be highly fibrogenic and carcinogenic. Even at mass dose levels of 4 mg/m^3 these materials were more pathogenic to rats than UICC Rhodesian chrysotile at 10 mg/m^3 (Davis *et al.* 1978). The studies were undertaken at this low dust concentration because it had proved extremely difficult to generate clouds of respirable dust from any of the WDC samples. In order to generate suitable clouds with these materials it was necessary to subject the yarn to a preliminary milling. Because of the low dust concentration, the high levels of pulmonary disease found in all experimental groups were unexpected. This raises the question of whether or not the reduced hazard suggested for the industrial use of WDC products because they generate little dust (Smither & Lewinsohn 1973) is offset to some degree by an increase in the harmfulness of the dust that is produced.

The results from this study do not, however, prove that the wet dispersed form of chrysotile is any more harmful to rats than other chrysotile materials, since normal chrysotile yarn subjected to a similar preliminary milling was found to be equally dangerous. It is interesting that UICC chrysotile has proved less active than these other chrysotile dusts but this may result from the preparation techniques used for all the UICC samples (Rendall 1980). The UICC standard reference samples of respirable asbestos fibres have amply fulfilled the purpose for which they were prepared and have proved extremely useful for many research purposes but there have been criticisms that they were too thoroughly milled and are too short to produce the maximum tissue response. Stanton *et al.* (1972, 1977) concluded that the most important factor in fibre pathogenesis was the number of long thin fibres in dusts and it would appear that the UICC samples have relatively few fibres of the most dangerous size.

The present study has confirmed that most chrysotile dusts produce very severe effects in rats. This has previously been noted in a number of publications where chrysotile has appeared more harmful than the amphiboles (Wagner *et al.* 1973, 1974; Davis *et al.* 1978). Some of this increased pathogenicity may be due to the fact that the chrysotile preparations had more long fibres than the amphiboles. However, some recent work in our Institute with a long amosite dust cloud indicates that other factors may be involved (Davis *et al.* 1985*b*). This amosite dust cloud had more fibres >5 μm in length (2000/ml by light microscopy) than the chrysotile and WDC products reported in the present paper, was used at a higher airborne mass concentration (10 mg/m^3) but produced fewer pulmonary tumours and less pulmonary fibrosis than these chrysotile preparations. The additional factor found with chrysotile dust may relate to the separation of inhaled fibres into their individual fibrils particularly in lung tissue where there are high concentrations of surfactant. This would result in a tissue dose of dangerous fibre units far higher than indicated by counts of airborne dusts.

The separation of chrysotile fibrils in tissue could explain the major anomaly that has been found between animal experimental studies and human epidemiology. While experimental studies almost without exception have reported chrysotile to be the most dangerous fibre tested, studies of asbestos workers have indicated that amphibole dusts, particularly crocidolite, are the most harmful to humans (Wagner *et al.* 1960, Weill *et al.* 1979). Studies of lung dust content in humans have shown that while chrysotile has usually formed the major part of any dust exposure, amphibole fibres predominate in the lungs at autopsy (Gylseth *et al.* 1983). It would appear very likely that while chrysotile can persist long enough in a short lived species like the rat to provoke a neoplastic response or pulmonary fibrosis, it may be removed from human lungs before

disease can develop. The separation of chrysotile bundles into individual fibrils is likely to be important in this process since these long but extremely thin structures might be expected to be more susceptible to dissolution.

The separation of WDC products into individual chrysotile fibrils within the tissues might explain the findings from the present inhalation studies using the sample of experimental WDC. The 4 mg/m^3 dust clouds of this material contained far fewer fibres than the other dust clouds studied but those present were longer and thicker. If all inhaled fibres that were deposited in the lung tissue separated into individual fibrils, then the number of these subunits would probably be similar with all the products tested and the similarity in the pathological response would not have been surprising.

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